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# Isolation and characterization of microsatellite DNA loci from *Sillago sihama*

YU-SONG GUO, ZHONG-DUO WANG, CHENG-ZHONG YAN, YU-LAN ZHANG, JIN-NAN ZHENG, YUAN-MIN XU, TAO DU and CHU-WU LIU\*

College of Fisheries, Guangdong Ocean University, Zhanjiang 524025, People's Republic of China

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### Introduction

The silver sillago, *Sillago sihama* Forsskål is naturally distributed in Indo–West Pacific: Red Sea and Knysna, South Africa to Japan and south to Australia (Wantiez 1993), and is common along beaches, sandbars, mangrove creeks and estuaries. Adults bury themselves in the sand when disturbed (McKay 1992), and feed mainly on polychaete worms, small prawns (*Penaeus*), shrimps and amphipods (Allen *et al.* 2002). This fish is found in all Chinese waters including East China Sea, Yellow Sea, Bohai Sea, South China Sea, and the Northern Bay. In China, it is a common marine food fish species, and the supply of this fish relies on capture from the wild. So far, studies of this species have focussed on ecology (Lee *et al.* 1981; Lu *et al.* 2008), morphology of early development (Wan 1996), discrimination of the genus *Sillago* with sagittal otolith shape (Pan and Gao 2010), and phylogenetic relationships of the genus *Sillago* (Xue *et al.* 2010; Gao *et al.* 2011). In the present study, we developed 66 microsatellite loci isolated from a dinucleotide-enriched genomic library of *S. sihama* using the fast isolation by AFLP of sequences containing repeats (FIASCO) protocol of Zane *et al.* (2002). These markers should provide a tool for assessing genetic diversity and population structure of wild stocks.

### Materials and methods

A sample of 25 individuals of *S. sihama* was randomly collected from coastal waters of Sanya, Hainan, People's Republic of China. Genomic DNA was extracted from the muscle tissue using a standard phenol–chloroform procedure (Sambrook *et al.* 1989). Total genomic DNA (about 500 ng)

derived were completely digested with *MseI* and then ligated to an *MseI* AFLP adaptor (MA-1: 5'-GAC GAT GAG TCC TGA G-3'; MA-2: 5'-TAC TCA GGA CTC AT-3'). A diluted digestion–ligation mixture (1:10) was amplified with adaptor-specific primers (MSEP: 5'-GAT GAG TCC TGA GTA A-3'). Amplified DNA fragments, with a size range of 200–1000 bp, were enriched for repeats by magnetic bead selection with a 5'-biotinylated (AC)<sub>15</sub> probes, respectively. Enriched fragments were amplified again with adaptor-specific primers. Polymerase chain reaction (PCR) products were purified using an EZNA Gel extraction kit (Omega Bio-Tek, Norcross, USA). Purified DNA fragments were ligated into the pMD-18T vector (TaKaRa), and transformed into DH5 $\alpha$  cells. Positive clones were tested by PCR using (AC)<sub>15</sub> and MSEP as primers, respectively. In total, 180 clones with positive inserts were sequenced with ABI PRISM 3730xl DNA sequencer. A total of 234 sequences were found to contain microsatellite repeats, and 124 of them were selected for designing locus-specific primers, using the oligo 6 software package (Molecular Biology Insights, Cascade, USA). Polymorphisms of all 59 microsatellite loci were assessed in 25 samples of *S. sihama* in Sanya. These microsatellite sequences have been deposited in GenBank (HM241218–HM241348).

The PCR reactions were performed in 10  $\mu$ L of reaction containing 30–50 ng genomic DNA, 0.5  $\mu$ M of each primers, 1  $\mu$ L 10 $\times$  PCR buffer (10 mM Tris-HCl (pH 8.3)), 50 mM KCl, 1.5 mM MgCl<sub>2</sub>, 0.2 mM each dNTPs and 1 U of *Takara Taq* polymerase. PCR runs began with an initial denaturation step at 94°C for 3 min followed by 30 cycles at 94°C for 30 s, 30 s at the annealing temperature ( $T_a$ ), 72°C for 45 s, and a final extension step at 72°C for 5 min. PCR products were separated on 8% (w/v) polyacrylamide gels using pBR322/*BsuRI* marker (MBI Fermentas, Shenzhen, China) by silver staining. Heterozygosity values and tests of Hardy–Weinberg equilibrium (HWE)

\*For correspondence. E-mail: liucw5206@163.com.

**Keywords.** FIASCO microsatellites; genetic diversity; *Sillago sihama*.

**Table 1.** Characterization of 66 microsatellite loci in *Sillago sihama*.

Locus	Primer sequence (5'-3')	T <sub>a</sub> (°C)	Size (bp)	Repeat motif	N <sub>a</sub>	N <sub>e</sub>	H <sub>o</sub>	H <sub>E</sub>	HWE P
<i>Ssi1</i>	F: ACTCAGGCTATGAAAACAAA R: TGATTCATCAAGTAAAGGAG	49	138–172	(tg) <sub>16</sub>	15	9.3	0.920	0.893	0.877
<i>Ssi2</i>	F: TGTGGTTCCCTGGTGG R: ATGACAAAGGCCGTTGAG	51	124–154	(ca) <sub>17</sub> N(ac) <sub>5</sub>	9	4.9	0.542	0.797	0.000*
<i>Ssi3</i>	F: TTAGGACAAATTCATGGTGGAT R: TCAGGCCGATATGGAGCT	52	128, 142	(gt) <sub>6</sub>	2	2.0	1.000	0.500	0.000*
<i>Ssi4</i>	F: CCTGCCCTCGCATCTGTA R: AGGAGATCAGCCACCAG	58	130–168	(tg) <sub>5</sub> N <sub>2</sub> (tg) <sub>3</sub> N <sub>2</sub> (tg) <sub>7</sub> N <sub>2</sub> (tg) <sub>4</sub>	5	3.4	0.920	0.703	0.049
<i>Ssi5</i>	F: TGCCCGCGTTGTAATCT R: TCGGAGGGAGCAAAGGAC	52	269–308	(ca) <sub>18</sub>	4	2.3	0.600	0.566	0.267
<i>Ssi6</i>	F: TCATCGTCACTGGTCCTT R: CAGCCACTGGAGACTAAA	49	156–188	(ac) <sub>17</sub>	10	4.9	0.652	0.798	0.000*
<i>Ssi7</i>	F: GAAAGCCATCACATCCTAAT R: CAACAACACTGCTCCCAAAA	50	158–206	(tg) <sub>17</sub> N <sub>15</sub> (tg) <sub>5</sub>	9	6.5	0.400	0.847	0.000*
<i>Ssi8</i>	F: CGCAGTGAACCCAAATGAGCA R: CGCAGTCTGGACGAAACG	58	160–192	(gt) <sub>9</sub> N <sub>2</sub> (gt) <sub>12</sub>	5	3.8	0.880	0.734	0.069
<i>Ssi9</i>	F: AGTGTCTGGAGCTAAGTGAT R: TGTCTGATGTTTATGTGGGT	49	148–188	(ca) <sub>5</sub> N <sub>2</sub> (ca) <sub>15</sub>	10	6.0	0.875	0.734	0.176
<i>Ssi10</i>	F: AGGAAACCCGAAATGTG R: CTTGGAGCTTGGCAGGAT	51	126–150	(tg) <sub>12</sub>	4	3.3	1.000	0.694	0.000*
<i>Ssi11</i>	F: AGCACCGTCAAATAAGC R: CCCAGCACCAATCAATA	54	186–222	(ac) <sub>10</sub> N <sub>2</sub> (ac) <sub>3</sub> N <sub>2</sub> (ac) <sub>6</sub>	9	6.7	0.600	0.850	0.015
<i>Ssi12</i>	F: TACGGCAGTCAGGGAAGT R: TACGCCAGGTCTGGAAC	61	176, 184, 192	(ca) <sub>6</sub>	3	2.2	0.360	0.553	0.000*
<i>Ssi13</i>	F: CGAGGAAGCCTCTGATGTGC R: GTGAGTCAGTGGAGACCAC	62	180–214	(tg) <sub>6</sub>	6	2.0	0.480	0.488	0.155
<i>Ssi14</i>	F: CTTTGGAGGAAAGTTGGG R: CATGATGGACGTTAATTGTA	51	134–200	(ca) <sub>17</sub>	12	8.8	0.955	0.886	0.001
<i>Ssi15</i>	F: ACGTGGGTAATCCACAIACA R: GAGGCAGAACTGAAGAAAA	53	174, 182, 192	(ca) <sub>5</sub> N <sub>3</sub> (ac) <sub>6</sub> N <sub>2</sub> (ac) <sub>7</sub> N <sub>2</sub> (ac) <sub>4</sub>	3	0.9	0.350	0.460	0.198
<i>Ssi16</i>	F: ATGCTGTCTAATCTAAAG R: TGAATAAATGGGTTACTGT	50	102–150	(ac) <sub>11</sub> (gcacac) <sub>2</sub> N <sub>5</sub> (ca) <sub>9</sub>	12	7.0	0.833	0.858	0.348
<i>Ssi17</i>	F: CCCGCATCTGTATTAGCA R: TCAAACGCAAGCCTATGT	53	192, 206, 220	(ca) <sub>18</sub> N(ac) <sub>37</sub>	3	2.2	1.000	0.555	0.000*
<i>Ssi18</i>	F: TTCTTCCACACAGCCTCAC R: CTTTATTACTGCGATGC	52	160–220	(ca) <sub>5</sub> N <sub>36</sub> (ac) <sub>16</sub>	8	5.2	0.778	0.809	0.288
<i>Ssi19</i>	F: TTTTCCCTTTTCATGCTGC R: AAAACCTTCATGGGTGAG	50	100	(ca) <sub>5</sub>	1	1.0	0.000	0.000	–
<i>Ssi20</i>	F: TCACCCATGAAGGGTTTG R: GCTGTCAGGAGATGTCGC	55	108	(ca) <sub>5</sub>	1	1.0	0.000	0.000	–
<i>Ssi21</i>	F: AGAGCGACATCTCCTGAC R: AGCTGTATCGTTGAACCC	54	192	(ca) <sub>7</sub>	1	1.0	0.000	0.000	–
<i>Ssi22</i>	F: GTGGCGATAGGACTTGTA R: GTCTTCCCTCCTCGTCAGT	49	360–308	(tctg) <sub>10</sub>	7	4.8	0.385	0.790	0.031

Table 1 (contd).

Locus	Primer sequence (5'-3')	$T_a$ (°C)	Size (bp)	Repeat motif	$N_a$	$N_e$	$H_0$	$H_E$	HWE P
<i>Ssi23</i>	F: GAGATTCAGTCAGCGGGATT R: TGTCAGGTGGCGGGTTTA	58	298–384	(ca) <sub>15</sub> N <sub>8</sub> (ca) <sub>7</sub>	16	10.7	0.750	0.906	0.097
<i>Ssi24</i>	F: TGCTCGGCATCTCCCTCT R: TGCCTCAGCCTGAACAAT	55	136–180	(ac) <sub>16</sub>	5	3.9	0.900	0.745	0.181
<i>Ssi25</i>	F: AAGCCTGCACCTAATGTTTC R: CTCCTCCTTTCATCCAIT	53	180–242	(ca) <sub>9</sub> N <sub>5</sub> (ac) <sub>9</sub>	9	5.0	0.846	0.802	0.103
<i>Ssi26</i>	F: GTCCCAACAGCTAGTGTT R: GTCTCCGGTTCAGATC	57	132–158	(ac) <sub>15</sub>	10	6.3	0.958	0.842	0.027
<i>Ssi27</i>	F: CCAAGCAAGAAAGGAAAC R: CAATCAGGGCCTGCAAAG	53	120–160	(ca) <sub>11</sub>	9	6.3	0.920	0.856	0.651
<i>Ssi28</i>	F: ATGTCGGAGGTTTGCTGT R: ACCACGCTAATGGGCTGT	50	162–226	(ca) <sub>13</sub> N <sub>2</sub> (ca) <sub>11</sub>	16	9.9	0.900	0.899	0.044
<i>Ssi29</i>	F: TCTGCTGCCAGTGGTTGT R: ATCTCAGTGCCATCTCCC	56	184, 202	(gt) <sub>15</sub>	2	1.7	0.600	0.420	0.032
<i>Ssi30</i>	F: ACAGTAGGCGCATCCACA R: TCTACATAGGACACGGACAG	53	166–190	(ac) <sub>11</sub> N <sub>5</sub> (ca) <sub>5</sub>	6	2.5	0.476	0.601	0.297
<i>Ssi31</i>	F: AAATACTTGTCAATCTCTGC R: TGAGACAGCATGGTTAG	51	260	(ac) <sub>7</sub> N <sub>5</sub> (ac) <sub>16</sub> N <sub>1</sub> (ca) <sub>12</sub> N <sub>1</sub> (ac) <sub>9</sub>	1	1.0	0.000	0.000	–
<i>Ssi32</i>	F: ACAAACAGCACAAAGGTG R: CAAATGATCAAATGGGTC	49	190–218	(ac) <sub>12</sub>	8	4.8	0.609	0.792	0.002
<i>Ssi33</i>	F: GATCTTCTCCACCACCGTCAG R: CAAATCCGAAAACATCACCC	49	252, 260, 270	(ca) <sub>6</sub> N <sub>18</sub> (ca) <sub>8</sub>	3	1.6	0.458	0.362	0.548
<i>Ssi34</i>	F: AAAGGCTCAGACAATCA R: CAACTCAAAGTGAACGAAA	53	182, 202	(ca) <sub>15</sub>	2	1.8	0.640	0.435	0.019
<i>Ssi35</i>	F: CATGGTACGGGTGATGAAGA R: AGGTGGACACGGTGAAGT	56	220–280	(tg) <sub>7</sub> N <sub>2</sub> (tg) <sub>5</sub> N <sub>4</sub> (ga) <sub>3</sub> N <sub>1</sub> (ag) <sub>4</sub> (ac) <sub>8</sub>	12	8.5	0.818	0.882	0.379
<i>Ssi36</i>	F: ACTGGCTAGATAGATGGG R: AGGTGTTCCGGTATAAAAT	49	182–196	(ac) <sub>8</sub>	4	3.5	1.000	0.718	0.000*
<i>Ssi37</i>	F: CATTACAGATTTCTCTTTC R: GACACTCTGGAGCCTAGCAG	52	230–270	(tg) <sub>9</sub> N <sub>1</sub> (gt) <sub>8</sub> N <sub>1</sub> (tg) <sub>5</sub>	7	4.5	0.833	0.780	0.363
<i>Ssi38</i>	F: GTCACCTTTGTCCTTACCTGT R: CTCAGTCATTTATACACCGT	53	238–310	(AC) <sub>8</sub>	6	4.1	0.100	0.759	0.000*
<i>Ssi39</i>	F: GTGCGTGCCTGTGCGGTTCA R: TGGCGGCTCTTTTCTTGT	55	226–292	(ca) <sub>7</sub> N <sub>1</sub> (ac) <sub>5</sub>	9	7.2	0.857	0.861	0.080
<i>Ssi40</i>	F: TCCCTTCCACTTGTTCCTC R: CCCACTCAGATGTTCCACC	58	286, 300, 318	(ac) <sub>8</sub> N <sub>15</sub> (ca) <sub>5</sub>	3	2.2	1.000	0.540	0.000*
<i>Ssi41</i>	F: GCTTTTATTTGGCGTCTTTA R: TGTGCCAGATGTCTCGTGT	54	126–168	(ac) <sub>5</sub> N <sub>2</sub> (ac) <sub>5</sub> N <sub>2</sub> (ac) <sub>5</sub>	9	8.3	0.760	0.880	0.292
<i>Ssi42</i>	F: TGTCTCCATTAGCCAAAAGTG R: GGAGGAAAAGAGGTCACAAA	56	152, 168, 174	(ac) <sub>8</sub>	3	2.6	0.958	0.609	0.000*
<i>Ssi43</i>	F: TGTCATATGTCCCAACCCA R: CTCGGAAGCCCAATCACTCA	58	196, 208, 226	(gt) <sub>7</sub> N <sub>1</sub> (ta) <sub>2</sub> N <sub>2</sub> (tg) <sub>6</sub>	3	1.2	0.200	0.185	0.958
<i>Ssi44</i>	F: AICCCGCCTTTGTTGTTCA R: TTCTGCTGTGCTGTGAAT	55	194, 202	(ac) <sub>7</sub>	2	1.2	0.167	0.153	0.656

Table 1 (contd).

Locus	Primer sequence (5'-3')	T <sub>a</sub> (°C)	Size (bp)	Repeat motif	N <sub>a</sub>	N <sub>e</sub>	H <sub>o</sub>	H <sub>E</sub>	HWE P
Ssi45	F: AAGATGAAAAACAGGGAGCA R: ATGTCGCACCTCAGGGGTAAA	57	132, 138	(ca) <sub>5</sub> N <sub>2</sub> (ca) <sub>3</sub>	2	1.6	0.417	0.375	0.586
Ssi46	F: TTGAACCTCCGTTATACAAAA R: AGAATAAGTAATCGCCAGTC	49	184	(ca) <sub>5</sub>	1	1.0	0.000	0.000	–
Ssi47	F: GCCTCTTTCCCTTACTTAG R: AACCAATGCCCTTTATTTAC	50	130–154	(ca) <sub>7</sub>	4	3.4	0.667	0.702	0.017
Ssi48	F: TAAACTCCCACTATCCTGTT R: CTTCCCTCAGTTTCTGCTTT	53	226, 234, 240	(aag) <sub>4</sub> N <sub>6</sub> (ga) <sub>3</sub> a <sub>8</sub> (gaaaaa) <sub>3</sub>	3	1.6	0.217	0.381	0.045
Ssi49	F: AGCTGTATTTAGTACCCCTC R: GATCCAGCACGAACCTTCTC	56	130, 152	(at) <sub>5</sub>	2	1.3	0.250	0.219	0.484
Ssi50	F: TCCCTCCCAAAATAGTGC R: AGCTGGATTCTTGTGAACT	55	128	(ac) <sub>5</sub>	1	1.0	0.000	0.000	–
Ssi51	F: TGTCACCAACTGGGAGGAG R: TTGGACCACTGAATGAGATT	51	108	(ac) <sub>5</sub>	1	1.0	0.000	0.000	–
Ssi52	F: GAACTGTAATTTGGTCTTT R: TATGTGGTGCATAGGTG	50	112, 116, 124	(ac) <sub>6</sub> N <sub>2</sub> (ac) <sub>6</sub>	3	1.8	0.417	0.434	0.000*
Ssi53	F: AGTTGACAGTAGCCCTCC R: TGTACCCGTTTCAGTTTGG	56	180–202	(ca) <sub>7</sub>	5	3.7	0.400	0.729	0.003
Ssi54	F: GGTGGACTGGTTTGTGTC R: GTGTCGGTTTGACGTTGC	51	152, 174	(ca) <sub>6</sub>	2	1.2	0.174	0.159	0.648
Ssi55	F: CTGGGCTGTAAACGTAATG R: GAAACTTTGACCTTCTCC	50	104, 120	(ca) <sub>7</sub>	2	1.4	0.360	0.295	0.272
Ssi56	F: CATTGAACATAAATTTGCTC R: AATATTCTGCTTTACACTCT	50	142, 148	(ca) <sub>6</sub> N(ac) <sub>5</sub>	2	1.8	0.440	0.449	0.922
Ssi57	F: TTTACCGGCTGGAGGAGG R: GTGCGGTTTGAGGGACGA	57	142, 176, 184	c <sub>7</sub> N <sub>3</sub> c <sub>8</sub> N <sub>8</sub> (ca) <sub>6</sub>	3	1.8	0.560	0.441	0.342
Ssi58	F: TAGAAATCAAAGAAATGCCACG R: GACCGAGTAACGAATACAAACCA	49	164–190	(ca) <sub>6</sub>	4	2.2	0.375	0.546	0.006
Ssi59	F: AGCTGGATTTCCCTGGGTT R: AGCGTCTTGGCATGTCGT	57	148–172	(ca) <sub>6</sub> N <sub>2</sub> (ca) <sub>11</sub>	4	2.2	0.760	0.551	0.007
Ssi60	F: TACTTCTCATTAGAGCCACG R: TCGGAGCTTGTGATTTGTA	52	190–230	(ga) <sub>9</sub> N <sub>12</sub> (ac) <sub>29</sub> N <sub>2</sub> (ag) <sub>6</sub>	8	6.3	0.950	0.840	0.007
Ssi61	F: TGGAGGTTGCTCGGTGTC R: AITTTGGATTTCTTTGGTTC	51	256–288	(ca) <sub>10</sub>	7	3.0	0.083	0.664	0.000*
Ssi62	F: ACTGACCTGCTCAATGACT R: CTTGGCACTGGCACTTCT	53	200–300	(ac) <sub>10</sub>	13	8.0	0.900	0.875	0.039
Ssi63	F: GCTGGAACGAAAGCGAAAA R: GCAAACCGCCACTGATGT	52	112, 116, 124	(ca) <sub>8</sub> N <sub>4</sub> (ac) <sub>6</sub>	3	1.5	0.385	0.328	0.864
Ssi64	F: TCTTTGCCCGTGAGGTAT R: TAGCCAATGAATGGTGAG	50	186, 192	(ca) <sub>7</sub>	2	1.2	0.000	0.147	0.000*
Ssi65	F: CCCTATCTGCTTACAC R: GATGATAGATCAGCCAGT	50	166, 176	(ca) <sub>5</sub>	2	1.2	0.214	0.191	0.653
Ssi66	F: TTTTCGAGGGCTTTAGGA R: AGTGCTGGAGTACAGGATTTT	50	250, 258, 268	t <sub>12</sub> N <sub>30</sub> (goga) <sub>4</sub> N <sub>27</sub> (ac) <sub>6</sub> N <sub>4</sub> (ac) <sub>5</sub>	3	2.2	0.500	0.554	0.547

\*P &lt; 0.0008; – means monomorphic.

were calculated using GENEPOP 3.4 package (10000 dememorizations, 100 batches, 5000 iterations per batch) (Raymond and Rousset 1995).

### Results and discussion

Seven loci (*Ssi19*, *Ssi20*, *Ssi21*, *Ssi31*, *Ssi46*, *Ssi50* and *Snu51*) were monomorphic. Descriptive statistics for the microsatellites are given in table 1. In 59 polymorphic loci, the numbers of alleles ranged from 2 to 16 and observed heterozygosity from 0.083 to 1.000. Fourteen microsatellite loci deviated significantly from HWE expectations including a Bonferroni correction (Bonferroni 1935) indicating either that null alleles were present or that the sample size was small. No significant disequilibrium within samples was found indicating that genotypes at pairs of microsatellite were randomly associated, and that the microsatellites were inherited in a Mendelian fashion.

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